AD_____

Award Number: DAMD17-03-1-0163

TITLE: A PSCA Promoter Based Avian Retroviral Transgene Model of Normal and Malignant Prostate

PRINCIPAL INVESTIGATOR: Doctor Robert Reiter

CONTRACTING ORGANIZATION: The University of California
Los Angeles, California 90024

REPORT DATE: April 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 2004	Annual (1 Apr 03-31 Mar 04)

4. TITLE AND SUBTITLE
A PSCA Promoter Based Avian Retroviral Transgene Model of Normal and Malignant Prostate

5. FUNDING NUMBERS
DAMD17-03-1-0163

6. AUTHOR(S)
Doctor Robert Reiter

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of California
Los Angeles, California 90024

E-Mail: rreiter@mednet.ucla.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates. All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The molecular and cellular origins of prostate cancer are poorly understood. Recent evidence from our laboratory suggests that prostate cancer may arise from a basal/luminal precursor cell marked by cell surface expression of PSCA. The evidence supporting this hypothesis is that (1) PSCA marks an intermediate cell population that co-expresses basal and luminal cell cytokeratins (2) this cell population is does not express p63 and is androgen receptor positive, all hallmarks of prostate cancer, and (3) PSCA is highly expressed in HGPIN and prostate cancer and in all animal models of prostate cancer. To test this hypothesis and to develop new models of prostate, we propose to determine whether delivery of oncogenes specifically to the PSCA positive cells of mouse prostate is sufficient to cause cancer. To accomplish this, we will develop a transgenic mouse model in which the retroviral receptor gene tva is expressed in the prostate under control of the PSCA promoter. Virus containing one or more oncogenes will be delivered to the prostate and the resulting phenotype characterized.

14. SUBJECT TERMS
Prostate cancer, prostate stem cell antigen, mouse model

15. NUMBER OF PAGES
7

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

The molecular and cellular origins of prostate cancer are poorly understood. Recent evidence from our laboratory suggests that prostate cancer may arise from a basal/luminal precursor cell marked by cell surface expression of PSCA. The evidence supporting this hypothesis is that (1) PSCA marks an intermediate cell population that co-expresses basal and luminal cell cytokeratins (2) this cell population is does not express p63 and is androgen receptor positive, all hallmarks of prostate cancer, and (3) PSCA is highly expressed in HGPIN and prostate cancer and in all animal models of prostate cancer. To test this hypothesis and to develop new models of prostate, we propose to determine whether delivery of oncogenes specifically to the PSCA positive cells of mouse prostate is sufficient to cause cancer. To accomplish this, we will develop a transgenic mouse model in which the retroviral receptor gene tva is expressed in the prostate under control of the PSCA promoter. Virus containing one or more oncogenes will be delivered to the prostate and the resulting phenotype characterized.

PROGRESS REPORT

Specific Aim 1. Establishment of a PSCA-tva transgenic line and characterization of the TVA positive cell population. (months 1-18)

This Aim has been completed.

Task 1. Multiple PSCA-tva transgenic lines were established. Expression of tva was confirmed in the prostates of all lines (Figure 1). TVA expression was confirmed in the prostate at the RNA and protein levels. As predicted, expression was also detected in bladder and stomach. Homozygous lines were established in order to speed generation of tva-positive offspring.

Task 2. Orthotopic delivery of virus was optimized and standardized, first using the marker gene GFP, and subsequently using luciferase. The latter marker enables us to monitor gene uptake into the prostate noninvasively using the CCD camera. (Figure 2) Orthotopic injection of virus into the dorsal lobe at 5 weeks of age results in reproducible uptake of virus and luciferase expression. Control mice are negative. Additional experiments are currently underway to determine if systemic administration of virus by intraperitoneal injection can improve prostatic uptake, since a limitation of the orthotopic approach is that virus uptake in the lateral and ventral lobes, which express high levels of tva, is suboptimal. To date, IP injection of luciferase-expressing virus does result in significant pelvic expression of luciferase. Whether expression is predominantly in the bladder or prostate or both will be determined by harvesting the organs of these mice and imaging them under the CCD camera.

Task 3 and 4. The PSCA/tva positive cells have furthermore been characterized to determine if they are equivalent to the human PSCA-positive prostate epithelial cells (i.e intermediate cells). This has been done in two ways. First, we tried to simply monitor the fate of GFP injected cells in vivo. This was unsuccessful, as the percentage of cells expressing GFP after orthotopic injection was too low to enable us to follow them over time. Second, we worked out conditions to culture the tva positive cells in vitro and then performed similar analyses to those done with human cells. These studies showed that the mouse tva positive cells were the same as the PSCA-positive human ones. Tva was expressed by only a subpopulation of mouse prostate epithelium in culture (figure 3). The percentage of tva positive cells increased with passaging. They were phenotypically distinct from other cells and appeared to form a second layer above the monolayer (figure 3). These were hallmarks of the human cells. Finally, we did an experiment in which we recombined the mouse prostate cells with urogenital sinus mesenchyme, inoculated the recombinant into the kidney capsule and harvested the resulting reconstituted tissue. When we stained the gland for tva, tva lit up what appeared to be developing glands in

which there was no lumen, while cells lining the mature glands were negative. We interpret these findings to be consistent with the notion that PSCA/tva is a marker of an intermediate precursor cell in the developing prostate. In Aim 2, we will test whether this cell type is transformable.

AIM 2: Induction of cancer using the oncogene polyoma virus middle T antigen (months 12-36)

Task 1-3. We have injected middle T antigen virus into more than 10 transgenic prostates. First, we successfully stained them by RNA in situ hybridization and could show that middle T was expressed by a subset of cells, as expected. Mice were then harvested at 1 year of age. Interestingly, and unexpectedly, we found that a majority of mice developed large hemangiomas (images of these are being generated for inclusion in next year's report). Parallel experiments with middle T injection into bladder resulted in the same—large hemangiomas. It turns out that endothelial cells are exquisitely sensitive to transformation by middle T, which leads to hemangioma formation. Although there is no detectable tva expression whatsoever in endothelium, it seems likely that the inflammation or trauma of orthotopic infection or very low-level expression of PSCA/tva by prostatic endothelium resulted in hemangioma formation. To resolve this potential problem, we will also attempt IP injection of middle T virus. In addition, we will move on to other oncogenes and combinations of oncogenes, which would be unlikely to cause an effect secondary to any low level background. To date, we have inoculated 10 mice with myc virus and 10 with activated Akt. Also, as planned in our study, we are crossing PSCA-tva mice with other knockouts. To date, we have crossed PSCA-tva with NKX3.1 knockouts and plan to cross with p27 knockouts as well. Finally, we are crossing PSCA-tva mice with conditional PTEN knockouts and will infect the bigenic offspring with cre expressing virus to inactivate PTEN in tva-positive cells. We hope that these solutions will overcome the problems with middle T virus and will result in models that are more analogous to human prostate cancer.

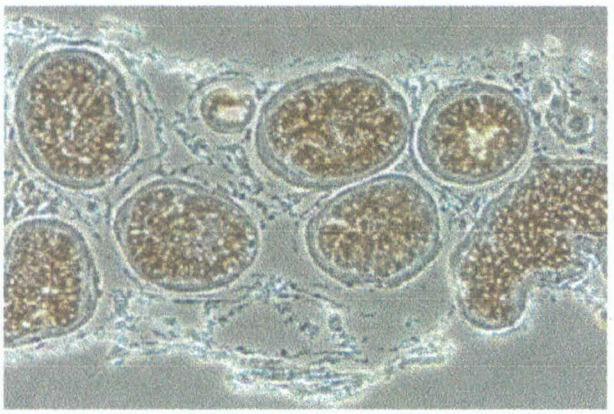

Figure 1: tva expression in dorsal lobe of mouse prostate. Note epithelial expression. No detectable expression is noted in surrounding stroma.

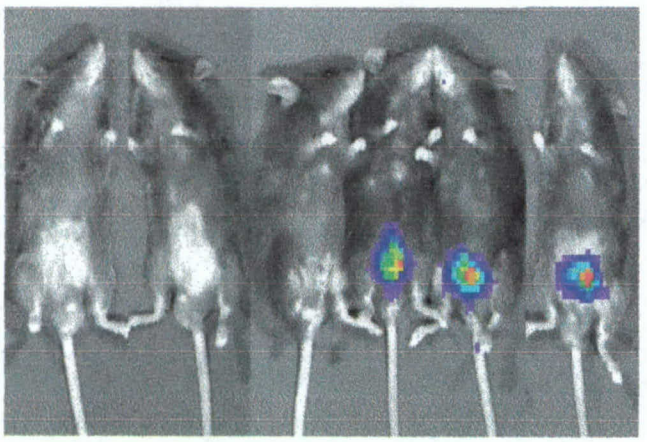

Figure 2: Expression of luciferase in the prostates of 3 PSCA-tva transgenic mice (right) compared with 3 wild-type mice. All were injected with virus at 5 weeks of age orthotopically into the prostate and imaged with the CCD camera at 8 weeks of age.

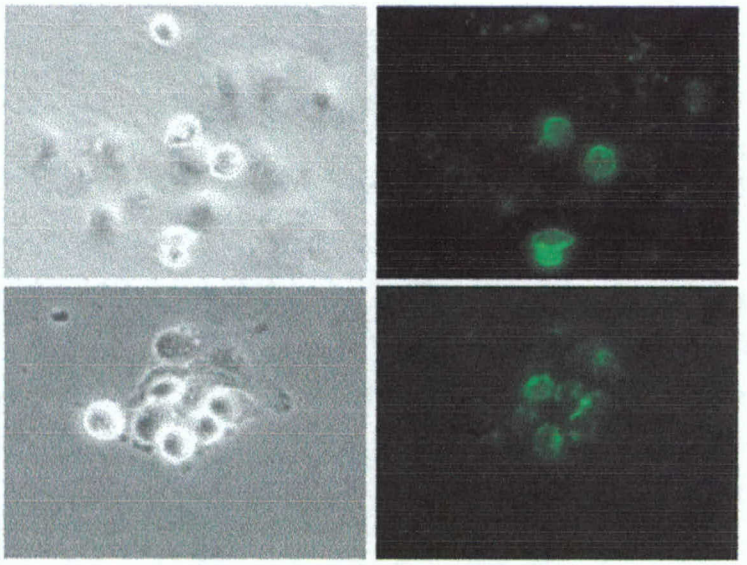

Figure 3: Mouse prostate epithelial cells in tissue culture (left). Immunofluorescent staining of tva positive cells (green cells on right). Note that tva positive cells are a subpopulation of the total cellular pool. Also note that the green cells represent those cells on the left, which appear more white and occupy a more superficial cell layer. These are hallmarks of PSCA cells in human prostate cultures as well.

KEY RESEARCH ACCOMPLISHMENTS OVER YEAR 1:

- Generation of PSCA-tva transgenic mice
- Successful orthotopic introduction of GFP and luciferase virus into the prostate, with ability to image the prostate
- In vitro cultivation of mouse prostate, demonstration of tva expression, and characterization of this cell population
- Reconstitution of PSCA-tva prostate epithelium with mesenchyme in mouse recombination model system.
- Inoculation of PSCA-tva mice with middle T virus and generation of hemangiomas
- Strategies to overcome the potential leakiness of the system as suggested by the middle T experiments.

REPORTABLE OUTCOMES

None

CONCLUSION: We have made significant progress over the past year. The major accomplishment has been the development of the model system, the demonstration that virus can be inoculated into and expressed by the prostate, and demonstration that tva positive cells are the predicted intermediate cells described in the human model. A number of obstacles were encountered with the middle T model, but we expect to surmount these obstacles over the remaining 2 years and to ultimately determine whether the model system can work.